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Probing the Structure-Function Relationships of Microbial Systems By High-Resolution *in vitro* Atomic Force Microscopy

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The elucidation of microbial surface architecture and function is critical to determining mechanisms of pathogenesis, immune response, physicochemical properties, environmental resistance and development of countermeasures against bioterrorist agents. We have utilized high-resolution *in vitro* AFM for studies of structure, assembly, function and environmental dynamics of several microbial systems including bacteria and bacterial spores. Lateral resolutions of ~2.0 nm were achieved on pathogens, *in vitro*. We have demonstrated[1-3], using various species of *Bacillus* and *Clostridium* bacterial spores, that *in vitro* AFM can address spatially explicit spore coat protein interactions, structural dynamics in response to environmental changes, and the life cycle of pathogens at near-molecular resolution under physiological conditions. We found that strikingly different species-dependent crystalline structures of the spore coat appear to be a consequence of nucleation and crystallization mechanisms that regulate the assembly of the outer spore coat, and we proposed a unifying mechanism for outer spore coat self-assembly. Furthermore, we revealed molecular-scale transformations of the spore coat during the germination process, which include profound, previously unrecognized changes of the spore coat. We will present data on the direct visualization of stress-induced environmental response of metal-resistant *Arthrobacter* oxydans bacteria to Cr (VI) exposure, resulting in the formation of a supramolecular crystalline hexagonal structure on the cell surface. At higher Cr (VI) concentrations the formation of microbial extracellular polymers, which cover microbial colony was observed. High-resolution visualization of stress-induced structures on bacterial surfaces builds a foundation for real time *in vitro* molecular scale studies of structural dynamics of metal-resistant bacteria in response to environmental stimuli. In the case of the bacterium *Chlamedia trachomatis,* we were able to identify surface exposed proteins versus proteins embedded in the outer membrane. These studies establish *in vitro* AFM as a powerful new tool capable of revealing pathogen architecture, structural dynamics and variability at nanometer-to-micrometer scales. This work was performed under the auspices of the U.S. Department of Energy by Univ. CA Lawrence Livermore National laboratory under contract number W-7405-ENG-48.

1. M.Plomp, T.J. Leighton, K.E. Wheeler and A.J. Malkin (2005). *Biophys. J.*, 88, 603-608. 2. M.Plomp, T.J. Leighton, K.E. Wheeler and A.J. Malkin (2005). Langmuir, 21, 7892. 3. M.Plomp, T.J. Leighton, K.E. Wheeler and A.J. Malkin (2005). Langmuir, 23, in press.